MDPI agronomy

VYTAUTAS MAGNUS UNIVERSITY AGRICULTURE ACADEMY

# Quality assessment of birch sap under ozone treatment

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## INTRODUCTION

Birch sap is a forest resource with a deep tradition of use in northern and eastern Europe [1]. Industrially obtained sap must be processed before the start of fermentation, because fermentation causes changes in color and odor, as well as the chemical composition of the sap [2,3]. Many alternative processing methods can be used to preserve the properties of fresh birch sap apart from pasteurization: microfiltration, treatment with ultrasound, UV radiation, magnetic fields, high pressure, and various combinations of these methods. These new innovative methods inactivate majority of microorganisms without the use of high temperatures which allows to preserve the original structure and properties of the product [2,3,4]. One such method could be the use of ozone gas. In the field of food processing, interest in ozone has grown rapidly in recent decades as consumer interest in nonthermal processing methods has increased [5,6]. Both aqueous and gaseous ozone have been universally recognized as safe to be used by the food industry (GRAS—Generally Recognized as Safe) by the U.S. Food and Drug Administration (FDA) [7,8]. According to the United States Department of Agriculture, food treated with ozone can be considered as "100% organic" or "organic" [8]. Due to its high oxidizing capacity, ozone inactivates most microorganisms, thus prolonging the shelf life and not damaging the product [6,10,11]. However, studies on the ozone concentrations used in sap treatment as well as the effect of ozone on changes in sap quality indicators are scarce [12]. The aim of this study is to determine the effectiveness of ozone at reducing microorganisms in birch sap and the effect of ozone on the composition of sap.

## MATERIALS AND METHODS

***Location.*** The sap was collected in a birch stand located in Sitkūnai forest of the Dubrava regional branch of the State Forest Enterprise (stand area 2.4 ha; Coordinates 55° 2'N; 23° 50'E). Soil type: Luvisol, temporarily flooded mineral soil stand parameters: age – 91 years; density – 308 trees $ha^{-1}$; average diameter at chest height – 39.4 ± 1.2cm; average height 25.0 ± 0.9m; wood volume 395 $m^3$ $ha^{-1}$.

The methodology and the course of the research are presented in Figure 1.

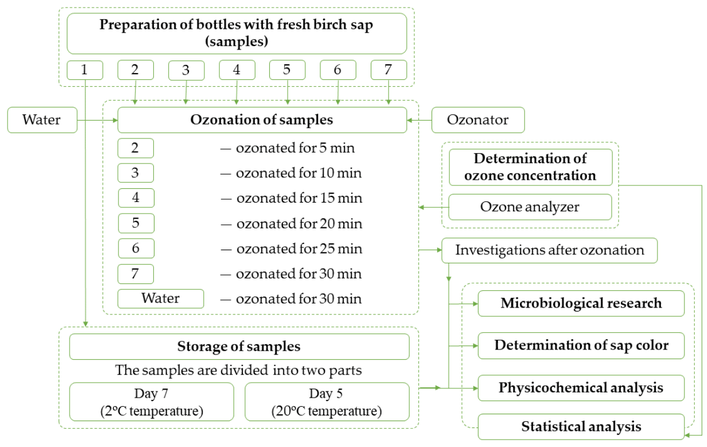


**Figure 1**. Evaluation of the influence of ozone on the biochemical composition of birch sap

## RESULTS

According to the study data (Figure 2), it can be seen that the distribution of ozone in water is more even and easier to distribute over the entire volume of water. After 5min, the ozone concentration in the water was 0.52 ± 0.028 mg $L^{-1}$ and no significant difference was observed between further ozonation intervals (Tukey HSD test, $p < 0.05$, $n = 6$). Observing the distribution of ozone concentration in the sap, it was observed that the change was not stable throughout the ozonation period of the sap. The average ozone concentration in the sap treated with ozone for 10 min was 0.99 ± 0.09 mg $L^{-1}$.

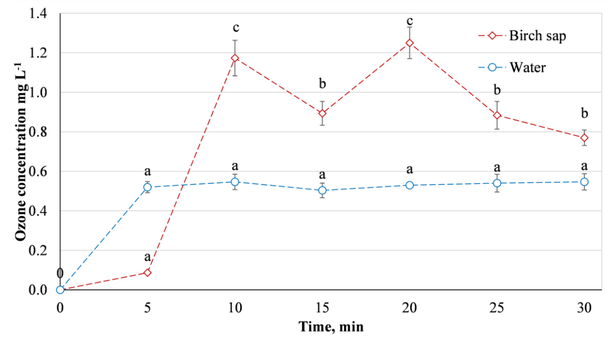


**Figure 2.** Changes of ozone concentration in sap and water. A Tukey HSD test was used to compare the means of ozone concentrations in sap and water ($p < 0.05$, $n = 6$).

The total number of bacteria, the number of lactic acid bacteria, and the number of yeasts and molds were chosen for the determination of the microbiological contamination of the sap.

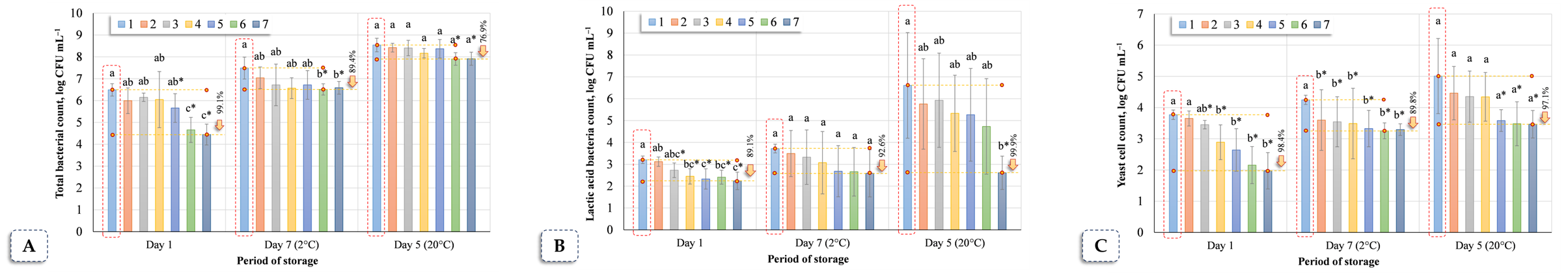


**Figure 3.** Changes in the total bacterial count (A), in the lactic acid bacteria count (B) and in the yeast colonies count (C) in birch sap: 1 (control) – not ozonated sap (initial); 2 – sap ozonated for 5 min; 3 – sap ozonated for 10 min; 4 – sap ozonated for 15 min; 5 – sap ozonated for 20 min; 6 – sap ozonated for 25 min; 7 – sap ozonated for 30 min. Significant, substantial differences are distinguished by letters (Tukey HSD test). Single letters indicate that there is no significant difference. Differences were considered significant when $p$ values were less than 0.05 ($n = 18$). In the Dunnett test, significant differences between the control group (No 1) and the treatment group are marked with an "*".

The influence of ozone on sap color, titratable and active pH acidity, Brix value, monosaccharides, sucrose, total sugars and ascorbic acid content was also evaluated.

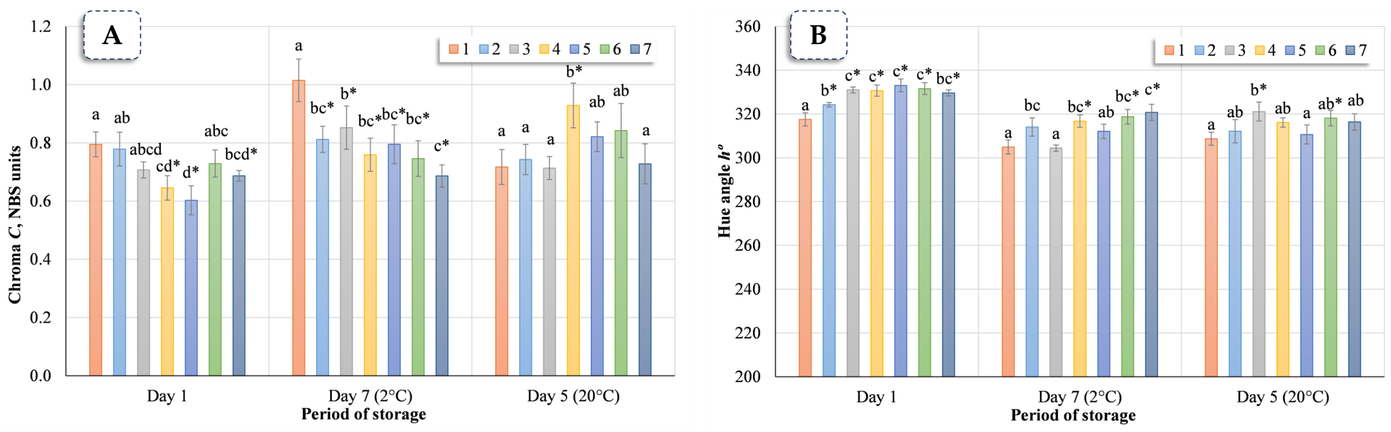


**Figure 4.** Changes in the chroma C (A) in the hue angle $h°$ (B) of birch sap during storage: 1 (control) – untreated sap (initial); 2 – sap ozonated for 5 min; 3 – sap ozonated for 10 min; 4 – sap ozonated for 15 min; 5 – sap ozonated for 20 min; 6 – sap ozonated for 25 min; 7 – sap ozonated for 30 min. Dunnett tests treat one group as a control (No 1) and compare all other groups against it. The difference between the control group and the other treatment groups is denoted by "*". Tukey HSD test – comparison of processing methods (marked in lower case letters). The mean difference is significant at the 0.05 level ($n = 18$)

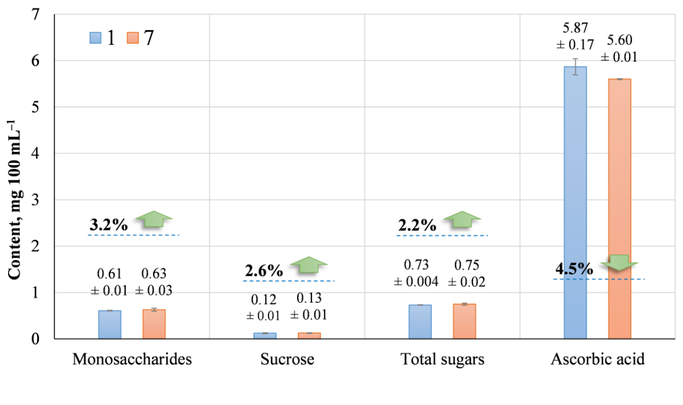


**Figure 7.** Comparison of components of fresh and ozonated for 30 min birch sap: 1 (control) – non-ozonated sap (initial); 7 – sap ozonated for 30 min. Data is presented as means ± standard deviations ($p < 0.05$, $n = 6$)

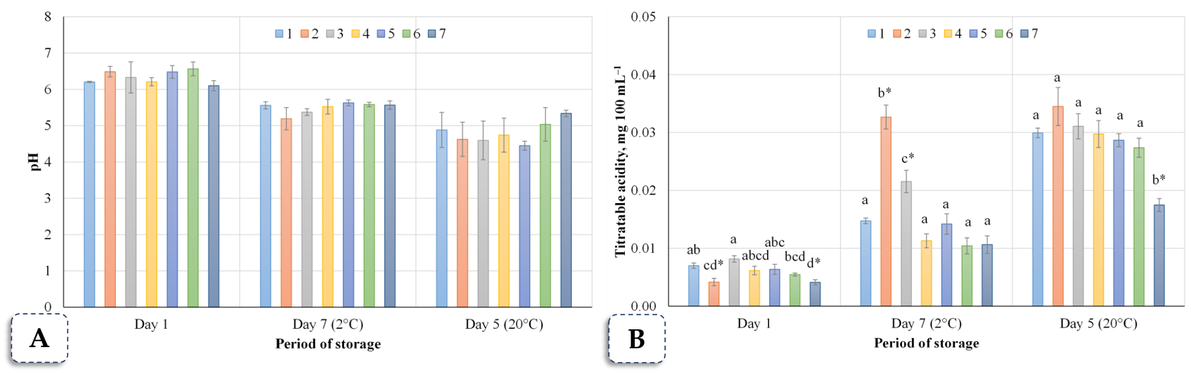


**Figure 5.** Variation of pH (A) and titratable acidity (B) of birch sap during storage: 1 (control) – not ozonated sap (initial); 2 – sap ozonated for 5 min; 3 – sap ozonated for 10 min; 4 – sap ozonated for 15 min; 5 – sap ozonated for 20 min; 6 – sap ozonated for 25 min; 7 – sap ozonated for 30 min. Significant differences ($p < 0.05$) were found according to Tukey HSD test (marked in lower case) and Dunnett tests (marked "*") ($n = 6$)

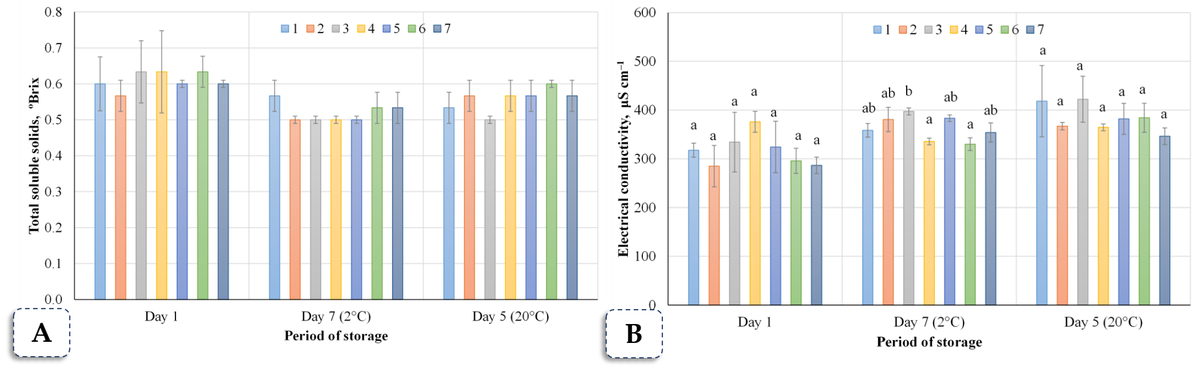


**Figure 6.** Variation of total soluble solids (A) and electric conductivity (B) of birch sap during storage: 1 (control) – not ozonated sap (initial); 2 – sap ozonated for 5 min; 3 – sap ozonated for 10 min; 4 – sap ozonated for 15 min; 5 – sap ozonated for 20 min; 6 – sap ozonated for 25 min; 7 – sap ozonated for 30 min. Significant differences ($p < 0.05$) were found according to Tukey HSD test (marked in lower case) ($n = 6$)

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## CONCLUSIONS

Rational use of ozonation on birch sap can reduce the number of microorganisms in the sap. For ozonation of sap using ozone with a concentration of 0.99±0.09 mg $L^{-1}$, the total reduction in bacterial colony count was up to 6.32–6.48 log CFU $ml^{-1}$, for lactic acid bacteria up to 2.46–3.15 log CFU $ml^{-1}$ and for yeast up to 3.16–3.76 log CFU $ml^{-1}$. The most significant effect of ozone was by ozonating the sap for 25 and 30 min. After 7 days (2°C) and 5 (20°C) days of storage, the numbers of microorganisms in the ozonated samples were observed to experience a statically significant decrease comparing to control. Ozone was found to affect the chroma and hue angle of birch sap color, although this difference was not visually noticeable. Ozonation was found to have no significant effect on pH, titratable acidity and °Brix values. After evaluating the effect of ozonation for 30 min on the monosaccharides, sucrose, total sugars and ascorbic acid in the juice, it was found that these values varied within the margin of error.

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